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(54) Title: NOVEL IMMUNOSUPPRESSIVE COMPOUNDS

(57) Abstract

This invention relates to anovel class of immuno-suppressive compounds having an affinity for the FK-506 binding protein (FKBP). Once bound to this protein, the immunosuppressive compounds inhibit the prolyl peptidyl cis-trans isomerase (rotamase) activity of the FKBP and inhibit T cell activation. As such, the compounds of this invention can be used as immunosuppressive drugs to prevent or significantly reduce graft rejection in bone marrow and organ transplantations and for use in the treatment of a wide variety of autoimmune diseases in humans and other mammals.

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NOVEL IMMUNOSUPPRESSIVE COMPOUNDS

Background of the Invention

Post operative graft rejections are a major complication affecting the success of bone marrow and organ transplantations. However, through the use of immunosuppressive drug therapy, graft rejection in organ transplantation can be significantly reduced.

A wide variety of diseases can be characterized as "autoimmune diseases". Such diseases are similar to graft rejection, except that the rejection is of self tissue. Immunosuppressive therapy can also be of use in preventing this inappropriate self rejection.

One widely accepted immunosuppressant for the prevention of graft rejection is cyclosporin A (CsA). It is a natural product of fungal metabolism and has been demonstrated to have potent immunosuppressive activity in clinical organ transplantations. Calne, R.Y. et al., Br. Med. J. 282:934-936 (1981); White, D.J.C. Drugs 24:322-334 (1982). Although CsA is widely used in immunosuppressant therapy, its usage (particularly in high dosage) is often accompanied by side effects which include nephrotoxicity, hepatotoxicity and other central nervous system disorders.

The following diseases have been treated with cyclosporin A with positive results, confirming the importance of the autoimmune component in these diseases and their effective treatment with compounds working by selective T-cell immune suppression similar to cyclosporin A.

1) Ophthalmology: Uveitis, Behcet's disease and Grave's ophthalmopathy.

Weetman, A.P. et al., Lancet 486-489 (1982). Grave's opthalmopathy.

Nussenblatt, R.B. et al., Lancet 235-238 (1983). Uveitis.

French-Constant, C. et al., Lancet 454 (1983). Behcet's disease.

Sanders, M. et al., Lancet 454-455 (1983). Behcet's disease.

Note: Cyclosporin A is currently approved in Japan for the treatment of Behcet's disease, the first autoimmune disease indication for this compound.

2) Dermatology: Various autoimmune skin diseases including psoriasis.

Zabel, P. et al., Lancet 343 (1984). Acute dermatomyositis.

van Joost, T. et al., Arch. Dermatol. 123:166-167 (1987). Atopic skin disease.

Appleboom, T. et al., Amer. J. Med. 82:866-867 (1987). Scleroderma.

Logan, R.A. and R.D.R. Camo, J. Roy. Soc. Med. 81:417-418 (1988). Eczema.

Griffiths, C.E.M. et al., Brit. Med. J. 293:731-732 (1986). Psoriasis.

Ellis, C.N. et al., J. Amer. Med. Assoc. 256:3110-3116 (1986). Psoriasis.

3) Hematology: Various diseases including anemia.

Toetterman, T.H. et al., Lancet, 693 (1984). Pure red cell aplasia (PRCA).

Stryckmans, P.A. et al., New Engl. J. Med. 310:655-656 (1984). Aplastic anemia.

Gluckman, E. et al., Bon Marrow Transplant 3

Suppl. 1, 241 (1988). Aplastic anemia.

4) Gastroenterology/Hepatology: Primary cirrhosis, autoimmune hepatitis, ulcerative colitis, Crohn's disease and other gastrointestinal autoimmune diseases.

Wiesner, R.H. et al., Hepatology 7:1025, Abst. #9, (1987). Primary biliary cirrhosis.

Hyams, J.S. et al., Gastroenterology 93:890-893 (1987). Autoimmune hepatitis.

Allison, M.C. et al., Lancet, 902-903 (1984). Crohn's disease.

Brynskov, J. et al., Gastroenterology 92:1330 (1987). Crohn's disease.

Porro, G.B. et al., Ital. J. Gastroenterol. 19:40-41 (1987). Ulcerative colitis.

5) Neurology: Amyotrophic lateral sclerosis (ALS, "Lou Gehrig's disease"), myasthenia gravis and multiple sclerosis.

Appel, S.H. et al., Arch. Neurol. 45:381-386 (1988). ALS.

Tindall, R.S.A. et al., New Engl. J. Med. 316:719-724 (1987). Myasthenia gravis.

Ann. Neurol. 24, No. 1, p. 169,m Abstract P174 (1988). Multiple sclerosis.

Dommasch, D. et al., Neurology 38 Suppl. 2, 28-29 (1988). Multiple sclerosis.

6) Nephrotic Syndrome: Nephrotic syndrome, membrano-proliferative glomerulonephritis (MPGN) and related diseases.

Watzon, A.R. et al., Clin. Nephrol. 25:273-274 (1986). Nephrotic syndrome.

Tejani, A. et al., Kidney Int. 33:729-734 (1988). Nephrotic syndrome.

Meyrier, A. et al., Transplant Proc. 20, Suppl. 4 (Book III), 259-261 (1988). Nephrotic syndrome.

LaGrue, G. et al., Nephron. 44:382-382 (1986). MPGN.

7) Rheumatoid Arthritis (RA)

Harper, J.I. et al., Lancet 981-982 (1984). RA

Van Rijthoven, A.W. et al., Ann. Rheum. Dis. 45:726-731 (1986). RA.

Dougados, M. et al., Ann. Rheum. Dis. 47:127-133 (1988). RA.

8) Insulin-Dependent Diabetes Mellitus (IDDM)

Stiller, C.R. et al., Science 223:1362-1367 (1984). IDDM.

Assan, R. et al., Lancet, 67-71 (1985). IDDM.

Bougneres, P.F. et al., New Engl. J. Med. 318:663-670 (1988). IDDM.

Diabetes 37:1574-1582 (1988). IDDM.

Many veterinary diseases are also characterized as autoimmune diseases. Autoimmune diseases such as those listed above have been observed in mammals. Papa, F.O. et al., Equine Vet. J. 22:145-146 (1990) infertility of autoimmune origin in the stallion; Gorman, N.T. and L.L. Werner, Brit. Vet. J. 142:403-410, 491-497 and 498-505 (1986) immune mediated diseases of cats and dogs; George, L.W. and S.L. White, Vet. Clin. North Amer. 6:203-213 (1984) autoimmune skin diseases in large mammals; Bennett, D., In. Pract. 6:74-86 (1984) autoimmune diseases in dogs; Halliwell, R.E., J. Amer. Vet. Assoc.

181:1088-1096 (1982) autoimmune diseases in domesticated animals.

The mechanism by which CsA causes immunosuppression has been established. In vitro, CsA inhibits the release of lymphokines, such as interleukin 2 (IL-2) [Bunjes, D. et al., Eur. J. Immunol. 11:657-661 (1981)] and prevents clonal expansion of helper and cytotoxic T cells [Larsson, E. J. Immunol. 124:2828-2833 (1980)]. CsA has been shown to bind the cytosolic protein, cyclophilin, and inhibit the prolyl-peptidyl cis-trans isomerase (PPIase) activity of that protein. Fischer, G. et al., Nature 337:476-478 (1989); Takahashi, N. et al., Nature 337:473-475 (1989). The PPIases may mediate T cell activation by catalyzing the rotomerization of peptide bonds of prolyl residues.

Recently, a second natural product isolated from Streptomyces, referred to as FK-506, has been demonstrated to be a potent immunosuppresive agent. Tanaka, H. et al., J. Am. Chem. Soc. 109:5031-5033 (1987). FK-506 inhibits IL-2 production, inhibits mixed lymphocyte culture response and inhibits cytotoxic T-cell generation in vitro at 100 times lower concentration than cyclosporin A. Kino, T. et al., J. Antibiot. 15:1256-1265 (1987). FK-506 also inhibits PPIase activity, but is structurally different from CsA and binds to a binding protein (FKBP) distinct from cyclophilin. Harding, M.W. et al., Nature 341:758-760 (1989); Siekierka, J.J., Nature 341:755-757 (1989).

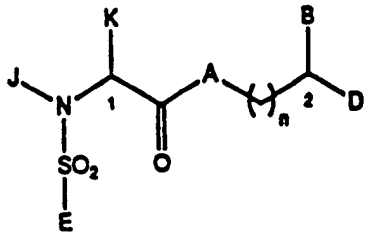
Summary of the Invention

This invention relates to a novel class of immunosuppressive c mpounds having an affinity for the FK-506 binding protein (FKBP). Once b und to this protein, the

immunosuppressive compounds inhibit the prolyl peptidyl cis-trans isomerase (rotamase) activity of the FKBP and lead to inhibition of T cell activation. The compounds of this invention can be used as immunosuppressive drugs to prevent or significantly reduce graft rejection in bone marrow and organ transplantations and in the treatment of autoimmune disease in humans and other mammals.

Detailed Description of the Invention

This invention relates to a novel class of immunosuppressive compounds having a sulfonamide substituent and which is represented by the formula I:



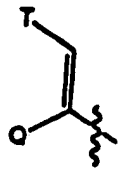
I

and pharmaceutically acceptable salts thereof,

wherein A is CH_2, oxygen, NH or N-(C1-C4 alkyl);

wherein B and D are independently Ar, hydrogen, (C1-C6)-straight or branched alkyl, (C1-C6)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl or alkenyl that is substituted with a (C5-C7)-cycloalkyl, (C1-C6)-straight or branched alkyl or alkenyl that is substituted with a (C5-C7)-cycloalkenyl, or Ar substituted (C1-C6)-straight or branched alkyl or alkenyl, wherein, in each case, one or two of the CH_2 groups of the alkyl or alkenyl chains may contain 1-2

heteroatoms selected from the group consisting of oxygen, sulfur, SO and SO_2 in chemically reasonable substitution patterns, or



provided that both B and D are not hydrogen;

wherein Q is hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted 5-7 membered cycloalkyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl, O-(C1-C4)-alkenyl and carbonyl;

wherein Ar is selected from the group consisting of phenyl, 1-naphthyl, 2-naphthyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, monocyclic and bicyclic heterocyclic ring systems with individual ring sizes being 5 or 6 which may contain in either or both rings a total of 1-4 heteroatoms independently selected from O, N and S; wherein Ar may contain one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, trifluoromethoxy, (C1-C6)-straight or branched alkyl, (C2-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl, O-(C2-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, 1,2-methylenedioxy, amino, carboxyl and phenyl;

wherein E is (C1-C6)-straight or branched alkyl, (C1-C6)-straight r branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or

branched alkyl or (C1-C4)-straight or branched alkenyl, [(C2-C4)-alkyl or (C2-C4)-alkenyl)]-Ar or Ar (Ar as described above);

wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylmethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an oxygen, sulfur, SO or SO_2 substituent therein; and

wherein n is 0-3.

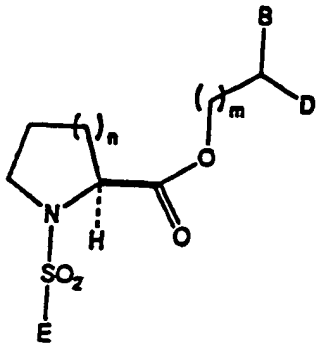
The stereochemistry at position 1 (Formula I) is (R) or (S), with (S) preferred. The stereochemistry at position 2 is (R) or (S).

The compounds of the present invention can be used in the form of salts derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salt with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-

containing groups can be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

Preferably, the compounds will have a molecular weight below about 750 atomic mass units (a.m.u.) and most preferably below about 500 a.m.u. Examples of compounds in which the J and K substituents are taken together to form a heterocyclic ring are shown in Table 1. As shown in Table 1, compound contains a five-membered heterocyclic ring system when n=1; and the compound contains a six-membered heterocyclic ring system when n=2.

TABLE 1: COMPOUNDS



No.	n	m	B	D	E
2	1	0	Hydrogen	Phenyl	Phenyl
3	1	0	Hydrogen	Phenyl	4-Methylphenyl
4	2	0	Hydrogen	Phenyl	4-Methylphenyl
5	2	0	Hydrogen	3-Phenylpropyl	4-Methylphenyl
6	2	0	Hydrogen	3-Phenylpropyl	4-Methoxyphenyl
7	2	0	Hydrogen	3-Phenylpropyl	2-Thienyl
8	2	0	Hydrogen	3-Phenylpropyl	2,4,6-Triisopropylphenyl
9	2	0	Hydrogen	3-Phenylpropyl	4-Fluorophenyl
10	2	0	Hydrogen	3-Phenylpropyl	Phenyl
11	2	0	Hydrogen	3-Phenylpropyl	3-Methoxyphenyl
12	2	0	Hydrogen	3-Phenylpropyl	2-Methoxyphenyl
13	2	0	Hydrogen	3-Phenylpropyl	3,5-Dimethoxyphenyl
14	2	0	Hydrogen	3-Phenylpropyl	3,4,5-Trimethoxyphenyl
15	2	0	Hydrogen	3-Phenylpropyl	Methyl
16	2	1	Hydrogen	3-Phenylpropyl	1-Naphthyl
17	2	0	Hydr gen	3-Phenylpropyl	8-Quinolyl

Table 1 (continued)

No.	n	m	B	D	E
18	2	0	Hydrogen	3-Phenylpropyl	1-(5-N,N-Dimethylamino)-naphthyl
19	2	0	Hydrogen	4-Phenoxyphenyl	4-Methoxyphenyl
20	2	0	Hydrogen	4-Phenoxyphenyl	4-Methylphenyl
21	2	0	Hydrogen	3-Phenoxyphenyl	2-Thienyl
22	2	0	Hydrogen	3-Phenoxyphenyl	8-Quinolyl
23	2	0	Hydrogen	3-Phenoxyphenyl	4-iodophenyl
24	2	0	Hydrogen	3-Phenoxyphenyl	2,4,6-Trimethylphenyl
25	2	0	Hydrogen	3-Phenoxyphenyl	Benzyl
26	2	0	Hydrogen	3-Phenoxyphenyl	2-Naphthyl
27	2	0	Benzyl	3-Phenylpropyl	4-Fluorophenyl
28	2	0	2-Phenylethyl	3-Phenylpropyl	4-Fluorophenyl
29	2	0	3-Phenylpropyl	3-Phenylpropyl	4-Methylphenyl
30	2	0	3-Phenylpropyl	3-Phenylpropyl	4-Nitrophenyl
31	2	0	3-Phenylpropyl	3-Phenylpropyl	3-Nitrophenyl
32	2	0	3-Phenylpropyl	3-Phenylpropyl	4-Chlorophenyl
33	2	0	3-Phenylpropyl	3-Phenylpropyl	4-Methoxyphenyl
34	2	0	3-Phenylpropyl	3-Phenylpropyl	4-Fluorophenyl
35	2	0	3-Phenylpropyl	3-Phenylpropyl	2-Thienyl
36	2	0	Hydrogen	3-Phenylpropyl	E-Styrenyl

The immunosuppressive compounds of this invention have an affinity for the FK-506 binding protein which is located in the cytosol of lymphocytes, particularly T lymphocytes. When the immunosuppressive compounds are bound to the FKBP, they act to inhibit the prolyl-peptidyl cis-trans isomerase activity of the binding protein and inhibit lymphocyte activation mediated by FKBP. One particular FK-506 binding protein has been identified by Harding, M.W. et al., Nature 341:758-760 (1989) and can be used as the standard by which to evaluate binding affinity of the compounds for FKBP. Compounds of this invention, however, may have an affinity for other FK-506 binding proteins. Inhibition of the prolyl peptidyl cis-trans isomerase may further be indicative of binding to an FK-506 binding protein.

Human FK-506 binding protein can be obtained as described by Harding, M.W. et al., Nature 341:758-760 (1989). Values for the apparent K_d can be determined from a competitive LH-20 binding assay performed as described by Harding et al., using 32-[1-^{14}C]-benzoyl FK-506 as a reporting ligand; or using [^{3}H]dihydro-FK-506, as described by Siekierka, J.J. et al., Nature 341:755-757 (1989). The binding affinities for two of the compounds of this invention for the FKBP are reported in the Examples Section. The data was obtained using the latter method, where the ability of an unlabeled compound to compete with the binding of [^{3}H]dihydro-FK-506 to FK-506 binding protein was measured.

The inhibition of the PPIase (rotamase) enzyme activity of the FKBP (apparent "Ki" values) can also be measured according to the meth ds d scribed by either

Harding, M.W. et al., Nature 341:758-760 (1989) or Siekierka, J.J. et al., Nature 341:755-757 (1989). The cis-trans isomerization of the proline-alanine peptide bond in a model substrate, N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide, is monitored spectrophotometrically in a coupled assay with chymotrypsin, which releases 4-nitroanilide from the trans form of the substrate. Fischer, G. et al., Nature 337:476-478 (1989). The inhibitory effect of the addition of different concentrations of inhibitor on the extent of the reaction is determined, and analysis of the change in first order rate constant as a function of inhibitor concentration yields an estimate of the apparent Ki value.

The compounds of the present invention can be further characterized in cellular biological experiments in vitro where their resemblance in function and use to cyclosporin A and to FK-506 is apparent. (See Table 2).

TABLE 2

Assays and IC_{50} Value for Drugs	Cyclosporin A	Rapamycin	FK-506
1) Human PBL + OKT3	<1μg/ml	<1μg/ml	<1μg/ml
2) T-Cell Hybridoma + TCR/CD2	<1μg/ml	<1μg/ml	<1μg/ml
3) Apoptosis	Blocks at 1μg/ml	Inactive at 1μg/ml	Blocks at 1μg/ml
4) CTLL Proliferation + IL-2	>>1μg/ml	≈0.01μg/ml	>>1μg/ml

1) Assay similar to Yoshimura, N. et al., Transplantation 47:356-359 (1989). Assay uses fresh human peripheral blood lymphocytes isolated by Ficoll-Hypaque density centrifugation, stimulated by the OKT3 antibody (anti-CD3) which stimulates via interaction with CD3. Stimulation is measured by incorporation of radioactive thymidine [(^{3}H)TdR] into proliferating cells, with an uninhibited control signal of 48,000-75,000 cpm. IC_{50} values are estimated from inhibitions of proliferation observed at various drug concentrations.

2) Assay similar to above, but using T-cell clone stimulated with antibody to the T-cell receptor (TCR) and antibody to CD2. Stimulation is measured by incorporation of radioactive thymidine [(^{3}H)TdR] into proliferating cells, with an uninhibited control signal of 23,000 cpm. IC_{50} values are estimated from inhibitions of proliferation observed at various drug concentrations.

3) Assay according to Shi, Y. et al., Nature 339:625-626 (1989). The assay uses a T-cell hybridoma similar to that described. The assay measures activation-induced (anti-CD3) cell death (evaluated by counting viable cells after staining as described) in a T-cell hybridoma that mimics the effect known to occur in immature thymocytes. The ability of cyclosporin A and FK-506 to inhibit this cell death is herein used as a sensitive indication of compounds with cyclosporin-like and/or FK-506-like mechanism of action. Note that the chemically related, but mechanistically distinct, immunosuppressant rapamycin is inactive in this assay.

4) Assay according to DuMont, F. et al., J. Immun l. 144:251-258 (1990). The assay measures the stimulati n f CTLL cells in resp nse t IL-2.

Proliferation is measured by incorpoation of (^{3}H)TdR. Immunosuppressants which work by a similar mechanism to cyclosporin A and FK-506 will not inhibit in this IL-2 driven process, since they function by the inhibition of production of endogenous IL-2. In this assay, exogenous IL-2 is provided to overcome this block. Note that the chemically related, but mechanistically distinct immunosuppressant, rapamycin, is active in this assay.

These assays and the ones set forth in the Example Section can be used to profile the cellular activity of the compounds of the present invention. It has been shown that the compounds of this invention resemble both cyclosporin A and FK-506 in its cellular activity, including immunosuppression, in contrast to the mechanistically dissimilar immunosuppressant agent rapamycin. Furthermore, the observed cellular activity is consistent quantitatively with the activity observed for FKBP binding and inhibition of PPIase (rotamase) activity.

Thus, the compounds can be used as immunosuppressants for prophylaxis of organ rejection or treatment of chronic graft rejection and for the treatment of autoimmune diseases.

The immunosuppressive compounds of this invention can be periodically administered to a patient undergoing bone marrow or organ transplantation or for another reason in which it is desirable to substantially reduce or suppress a patient's immune response, such as in various autoimmune diseases. The compounds of this invention can also be administered to mammals other than humans f r treatment of various mammalian autoimmune diseases.

The novel compounds of the present invention possess an excellent degree of activity in suppression of antigen-stimulated growth and clonal expansion of T-cells, especially those T-cells characterized as "helper" T-cells. This activity is useful in the primary prevention of organ transplant rejection, in the rescue of transplanted organs during a rejection episode, and in the treatment of any of several autoimmune diseases known to be associated with inappropriate autoimmune responses. These autoimmune diseases include: uveitis, Behcet's disease, Graves ophthalmopathy, psoriasis, acute dermatomyositis, atopic skin disease, scleroderma, eczema, pure red cell aplasia, aplastic anemia, primary cirrhosis, autoimmune hepatitis, ulcerative colitis, Crohn's disease, amyotrophic lateral sclerosis, myasthenia gravis, multiple sclerosis, nephrotic syndrome, membranoproliferative glomerulonephritis, rheumatoid arthritis and insulin-dependent diabetes mellitus. In all of the above-listed autoimmune diseases, treatment is effective to reduce the symptoms and slow progression of the disease. In the case of insulin-dependent diabetes mellitus, treatment as described below is most effective when instituted before the complete cessation of natural insulin production and transition to complete dependence on external insulin.

For these purposes the compounds of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous, intraven us, intramuscular, intrasternal and intracranial inj ction r infusi n techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectible aqueous or oleagenous suspension. This suspension may be formulated according to techniqu s known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids such as oleic acid and its glyceride derivatives find use in the preparation of injectables, as do natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant such as Ph. Helv or similar alcohol.

The compounds may be administered orally, in the form of capsules or tablets, for example, or as an aqueous suspension or solution. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The compounds of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The compounds of this invention may also be administered topically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application, including autoimmune diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas.

For ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively for the ophthalmic uses, the compounds may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds can be formulated in a suitable ointment containing the compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated in a suitable lotion or cream containing the active compound suspend d r dissolved in, f r example, a

mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation.

Dosage levels on the order of 0.01 to 100 mg/kg per day of the active ingredient compound are useful in the treatment of the above conditions. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

It is understood, however, that a specific dose level for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination and the severity of the particular disease being treated.

The compound can also be administered in combination with a steroid, such as methyl prednisalone acetate, for additional immunosuppressive effect. The steroid is administered orally, intravenously, rectally, topically or by inhalation. Dosages (based upon methyl prednisalone acetate) of 0.1-5 mg/kg/day may be employed. An initial loading dose of 100-500 mg may be employed. Steroid doses may be decreased with time from the higher toward the lower doses as the clinical situation indicates.

The compounds can be administered with other immunosuppressant drugs, such as rapamycin, azathioprine, 15-deoxyspergualin, cyclosporin, FK-506 or combinations of these, to increase the immunosuppressive effect. Administration of cyclosporin and FK-506 together should be avoided due to contraindications reported resulting from coadministration of these immunosuppressants. The dosage level of other immunosuppressant drugs will depend upon the factors previously stated and the immunosuppressive effectiveness of the drug combination.

OKT3, which is a murine monoclonal antibody to CD3 surface antigen of human T lymphocytes, can also be coadministered intravenously with compounds of the present inventions for rescue and reversal of acute allograft rejections, particularly in renal transplantations.

The invention will be further illustrated by way of the following examples, which are not intended to be limiting in any way.

EXAMPLES

General

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 500 MHz on a Bruker AMX 500. Chemical shifts are reported in parts per million (δ) relative to Me_4Si (δ 0.0). Analytical high performance liquid chromatography (HPLC) was performed on either a Waters 600E or a Hewlett Packard 1050 liquid chromatograph.

EXAMPLE 1

Synthesis of (S)-3-Phenylbutyl N-(4-methylsulfonyl)-pipecolate (5)

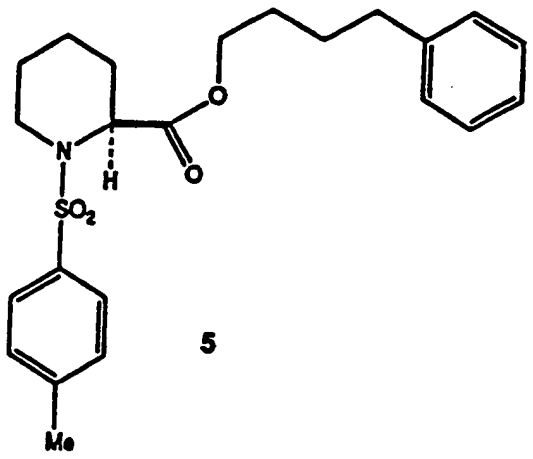
(S)-3-Phenylbutyl Pipecolate (38)

To a slurry of 5.0 g (17.9 mmol) of the tartrate salt of (S)-pipecolic acid (Egbertson, M. and S.J. Danishefsky, J. Org. Chem. 54:11 (1989)) in 50 mL of dry benzene was added 13.8 g (89.5 mmol) of 4-phenyl-1-butanol (Aldrich Chemical Co.) and 3.76 g (19.8 mmol) of p-toluenesulfonic acid monohydrate and the resulting mixture was heated at reflux overnight under a Dean Stark trap. The resulting homogeneous solution was concentrated, dissolved into 100 mL of 4:1 ether:ethyl acetate and extracted with 0.5 N HCl. The acidic aqueous layer was washed with 100 mL of 4:1 ether:ethyl acetate, basified by the addition of 8.0 mL of 30% NH_4OH and was then extracted into ethyl acetate. The combined organic extracts were washed with brine, dried over $MgSO_4$ and concentrated to give 4.5 g of the free amine (38) as an oil. ^1H NMR consistent with structure.

(S)-3-Phenylbutyl N-(4-methylsulfonyl)pipecolate (5)

To a solution of 30 mg (0.12 mmol) of the free amine (38) in 0.5 mL of CH_2Cl_2 was added 22 μL (0.13 mmol) of diisopropylethylamine and 24 mg (0.13 mmol) of p-toluenesulfonyl chloride and the resulting mixture was allowed to stir at room temperature for 1 hr. The reaction mixture was concentrated and flash chromatographed (elution with a gradient of 5% ethyl acetate in hexan to 100% thyl acetate) t give 29 mg f

the sulfonamide (5) as an oil. The compound is illustrated below. ^{1}H NMR (500 MHz $CDCl_3$) δ 7.62 (d), 7.29-7.09 (m), 4.71 (br d), 4.02-3.95 (m), 3.91-3.83 (m), 3.71 (br d), 3.21-3.12 (m), 2.58 (t), 2.46 (s), 2.10 (br d), 1.75-1.66 (m), 1.65-1.40 (m), 1.30-1.15 (m).



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EXAMPLE 2

Synthesis of (S)-1,7-Diphenyl-4-heptanyl N-(4-methoxysulfonyl)pipecolate (33)

4-Phenyl-1-butyraldehyde (39)

To a solution of 3.2 mL (20.8 mmol) of 4-phenyl-1-butanol (Aldrich Chemical Co.) in 20 mL of CH_2Cl_2 at 0°C was added 3.2 g of powdered 3 A molecular sieves and then 5.37 g (24.9 mmol) of pyridinium chlorochromate (PCC). The resulting suspension was stirred at 0°C for 1 h at which time an additional 2.16 g (10.0 mmol) of PCC was added and the reaction mixture was warmed to room temperature. After stirring at ambient temperature for 0.5 h, the reacti n mixture was diluted

with ether and filtered through celite to give 2.5 g of the crude product. Flash chromatography (elution with 5% ethyl acetate in hexane) yielded 700 mg of the aldehyd (39). ^{1}H NMR consistent with the product.

3-Phenyl-1-propylmagnesium bromide (40)

To a suspension of 736 mg (30.3 mmol) of magnesium turnings in 50 mL of THF at room temperature was added 50 μL of 1,2-dibromoethane followed by the dropwise addition of 5.5 g (25.1 mmol) of 1-bromo-3-phenylpropane (Aldrich Chemical Co.). After stirring at room temperature for 0.5 h the supernatent was transferred via cannula to a 100 mL storage vessel and subsequently used as a 0.5 M THF solution of the Grignard reagent (40).

1,7-Diphenyl-4-heptanol (41)

To a solution of 700 mg (4.7 mmol) of 4-phenyl-1-butanal (39) in 5.0 mL of THF at 0°C was added 10.0 mL (5.0 mmol) of 3-phenyl-1-propylmagnesium bromide (40) and the resulting mixture was stirred at 0°C for 0.5 h. The mixture was then quenched by the dropwise addition of saturated NH_4Cl and diluted with ether. The phases were separated and the organic layer was washed with water and brine and then dried over $MgSO_4$. Concentration gave 1.12 g of the alcohol (41) as an oil. The ^{1}H NMR spectrum of this compound ($CDCl_3$) was consistent with the structure.

(S)-Boc-Pipecolyl-1,7-diphenyl-4-heptanyl ester (42)

To a solution of 164 mg (0.72 mmol) (S)-Boc-L-pipecolic acid in 5.0 mL of CH_2Cl_2 at room temperature was added 174 mg (0.65 mmol) of alc hol 41, 140 mg (0.72

mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and a catalytic amount of N,N-dimethylaminopyridine (DMAP). The reaction mixture was stirred at ambient temperature for 0.5 h and then applied directly to a silica gel column. Elution with 10% ethyl acetate in hexane afforded 76.2 mg of the ester (42) as an oil. ^{1}H NMR consistent with product.

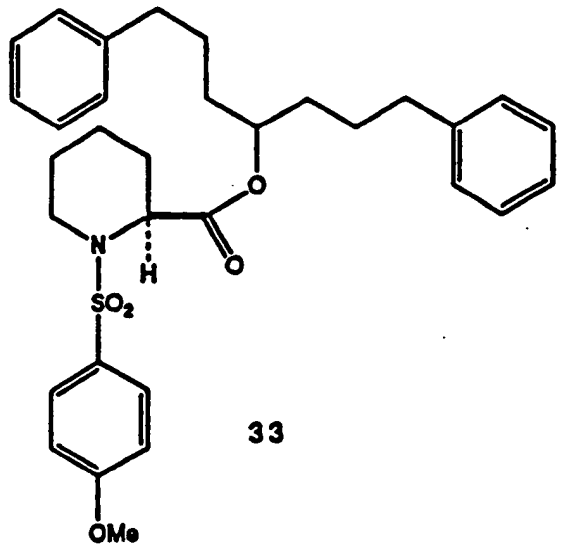
(S)-1,7-Diphenyl-4-heptanylpipecolate (43)

To a solution of 47 mg (0.10 mmol) of (42) in 1.0 mL of CH_2Cl_2 at ambient temperature was added 1.0 mL of trifluoracetic acid. After stirring at room temperature for 0.5 h, the resulting solution was neutralized by the dropwise addition of saturated K_2CO_3. The layers were separated and the organic phase was washed with water, dried over $MgSO_4$ and concentrated to yield 23 mg of the amine (43) as an oil. ^{1}H NMR consistent with structure.

(S)-1,7-Diphenyl-4-heptanyl
N-(4-methoxysulfonyl)pipecolate (33)

To a solution of 12.1 mg (0.031 mmol) of the free amine (43) in 1.0 mL of CH_2Cl_2 was added 6.0 μL (0.035 mmol) of diisopropylethylamine and 7.1 mg (0.034 mmol) of 4-methoxybenzenesulfonyl chloride and the resulting mixture was allowed to stir at room temperature for 1 h. The reaction mixture was concentrated and flash chromatographed (elution with 5% ethyl acetate in hexane) to give 10.5 mg of the sulfonamide (33) as an oil. The structure of (33) is shown below. ^{1}H NMR (500 MHz $CDCl_3$) δ 7.43-7.33 (br d), 7.09-6.97 (m), 6.96-6.83 (m), 6.54 (br d), 4.61 (br d), 4.48 (s), 3.58-3.45 (m), 3.01-2.92

(br t), 2.39-2.25 (br t), 1.88 (br d), 1.59-1.47 (br d), 1.46-1.19 (m), 1.06-0.95 (m).



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EXAMPLE 3

Cell Source and Culture

Fresh peripheral blood lymphocytes (PBLs) from LeukoPak cells or whole blood from random normal blood donors (tested HIV-negative and hepatitis negative) are isolated and separated by density centrifugation over Histopaque 1077 (Sigma Chemical Co., St. Louis, MO). The murine CTLL cytotoxic T cell line and the human Jurkat T cell line are from ATCC (CTLL-2 ATCC TIB214, JURKAT CLONE E6-1 ATCC TIB152). The human allogeneic B cell lines used for activation of the fresh PBLs are EBV-transformed lymphocytes from normal healthy adult donors with two completely different HLA haplotypes. All cell lines were routinely tested f r th presence of Mycoplasma

contamination using the Gibco Mycotect test kit and are Mycoplasma-free. Culture medium consists of RPMI 1640 (Gibco, Grand Island, NY) containing penicillin (50 U/ml) and streptomycin (50 μg/ml), L-glutamine 2 mM, 2 mercaptoethanol (5×10^{-5}), 10% heat-inactivated FCS and 10 mM HEPES.

Compound Solutions and Titrations

All chemical stocks were dissolved in DMSO. Titrations of compounds were made into the medium the individual assay was carried out in, i.e., complete RPMI or HB 104 for final diluted concentrations, using multiple three-fold dilutions from 1 μM or 10 μM stock solutions.

MTT Assay

The MTT assay is a colorimetric technique to determine the toxicity of the compounds on growing lymphoid and non-lymphoid cell lines based on reduction of the tetrazolium salt by intact mitochondria (Mossman, T., J. Immunol. Methods 65:55 (1983)). Cell viability in the presence or absence of different concentrations of test compounds in serum-free medium (HB 104, HANA Biologic, Inc.) was assessed using MTT (3-[4,5-dimethyl-thiazoyl-2-yl]2,5-diphenyl-tetrazolium bromide). At 4 h before the end of the 3-day toxicity assay culture period, 20 μl of MTT dye (5 mg/ml in pH 7.2 PBS) were added to each microtiter well. At the end of the incubation time, most of the culture media was carefully aspirated out of each well. Then 100 μl of acidified is propyl alc h l (0.04 N HCl) was added to

solubilize the dye and optical density is read at 570 nm minus OD at 630 nm (Molecular Devices Thermomax plate reader and Softmax software program, Menlo Park, CA). Results were compared with mean OD in controls (medium with no drugs) and doses causing 50% toxicity (TC_{50}) w re calculated.

Mitogenesis Assays ("PMA" and "OKT3")

The inhibitory effect of test compounds on the proliferation of human PBLs in response to mitogens (Waithe, W.K. and K. Hirschhorn, Handbook of Experimental Immunology, 3d Ed. Blackwell Scientific Publications, Oxford (1978); Mishell, B.B. and S.M. Shiigi, Selected Methods in Cellular Immunology W.H. Freeman and Co., San Francisco, CA (1980)) was assessed by stimulation of 5 x 10^4 cells with OKT3 (10^{-4} dilution final) or PMA (10ng/ml) plus ionomycin (250 ng/ml) in the presence or absence of different concentrations of test compounds and control drugs (CsA, FK506, Pagamycin) in final volume of 200 μl per well in 96 well round bottomed plates. After 48 h incubation (37°C, 5% CO_2), cells were pulsed with 1 μCi of ^{3}H-thymidine, harvested 24 h later with a Tom Tek cell harvester, and counted in LKB β-scintillation counter. Compounds were evaluated on their ability to inhibit proliferation of human peripheral blood lymphocytes. Results (cpm) were compared with controls with medium alone, and concentrations causing 50% reduction in counts (IC_{50}) were calculated.

MLR Bioassays ("LB" and "JVM")

Antigen activated proliferation of PBLs in a primary mixed lymphocyte reaction was assessed in the presence r absence of different concentrations of tested compounds and control drugs. 5 x 10^4 fresh PBLs were stimulated with 5 x 10^3 of Mitomycin C treated-allogeneic EBV-transformed β-lymphoblastoid cells, LB and JVM, in a final volume of 200 μl per well in 96-well round-bottomed plates (Mishell, B.B. and S.M. Shiigi, Selected Methods in Cellular Immunology W.H. Freeman and Co., San Francisco, CA (1980); Nelson, P.A. et al., Transplantation 50:286 (1990)). Cultures were pulsed on day 6, harvested 24 h later and counted as in previous section. The compounds were evaluated on their ability to inhibit proliferation in a mixed lymphocyte reaction.

IL-2 Microassay ("CTLL")

To determine if test compounds inhibit the later T cell activation process of cytokine utilization, the proliferative response of the IL-2 dependent CTLL-20 murine T cell line (ATCC) was assessed (Gillis, S. et al., J. Immunology 120:2027 (1978)). CsA and FK506 inhibit the production of IL-2 by activated T cells, whereas Rapamycin interferes with the utilization of IL-2. Rapamycin thus inhibits IL-2 dependent proliferation of the CTLLs, and CsA and FK506 do not (Dumont, F.J. et al., J. Immunology 144:251 (1990)). 3 x 10^3 CTLLs were exposed to different concentrations of test compounds and control drugs in the presence of 1 U/ml of human recombinant IL-2 (Genzyme, rIL-2) for 24 h. F ur h after adding drugs, cells were pulsed with 1 μCI

of 3H-thymidine, incubated for an additional 20 h (37°C, 5% CO_2), and then harvested and counted as previously described.

Results

The results of these assays are tabulated in Table 3 below.

TABLE 3: ASSAY RESULTS

No.	Ki(nm)	Kd(nm)	PMA	OKT3	LB	JVM	CTLL
2	16,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
3	2,500	ND	>10,000	>10,000	>10,000	>10,000	>10,000
4	1,500	1000	>10,000	>10,000	>10,000	>10,000	>10,000
5	130	ND	5,000	3,500	>10,000	>10,000	7,500
6	180	ND	>10,000	>10,000	9,000	4,500	1,000
7	200	ND	>10,000	8,000	>10,000	>10,000	>10,000
8	2,400	ND	>10,000	>10,000	>10,000	>10,000	>10,000
9	100	90	>10,000	>10,000	>10,000	>10,000	>10,000
10	200	ND	>10,000	>10,000	>10,000	>10,000	>10,000
11	2,200	ND	>10,000	>10,000	8,000	>10,000	>10,000
12	8,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
13	3,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
14	240	ND	>10,000	8,000	>10,000	>10,000	>10,000
15	6,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
16	900	ND	>10,000	>10,000	>10,000	>10,000	>10,000
17	>100,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
18	2,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
19	5,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
20	2,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
21	500	ND	>10,000	>10,000	>10,000	>10,000	>10,000

TABLE 3: ASSAY RESULTS (continued)

No.	Ki(nm)	Kd(nm)	PMA	OKT3	LB	JVM	CTLL
22	>100,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
23	2,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
24	900	ND	>10,000	>10,000	4,000	>10,000	>10,000
25	1,900	ND	9,000	>10,000	>10,000	>10,000	>10,000
26	2,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
27	12,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
28	1,500	ND	>10,000	>10,000	>10,000	>10,000	>10,000
29	1,700	ND	>10,000	>10,000	>10,000	>10,000	>10,000
30	3,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
31	6,000	ND	>10,000	8,000	>10,000	>10,000	>10,000
32	8,000	ND	>10,000	>10,000	>10,000	>10,000	>10,000
33	5,000	ND	7,500	9,500	7,500	>10,000	>10,000
34	700	ND	>10,000	>10,000	>10,000	>10,000	>10,000
35	2,500	ND	>10,000	>10,000	ND	>10,000	9,000
36	400	ND	>10,000	>10,000	>10,000	>10,000	>10,000

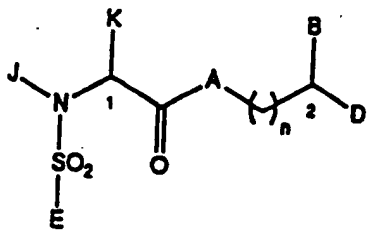
K_i - inhibition of FKBP rotamase activity
K_D - binding to FKBP
PMA and OKT3 - mitogens used to stimulate proliferation of human peripheral blood lymphocytes (PBC). Compounds are evaluated on their ability to inhibit proliferation.
LB and JVM - human viral-transformed B lymphoblastoid cell lines stimulated to proliferate in a mixed lymphocyte reaction (MLR). The compounds are evaluated on their ability to inhibit this proliferation.
CTLL - inhibition of proliferation of cytotoxic T cells stimulated by IL-2.
ND - not determined.

Equivalents

Those skilled in the art will recognize, or be able to ascertain, using n more than r utine experimentation, many equivalents to the specific embodim nts of the invention described herein. Such equivalents are intended to be encompassed by the following claims:

CLAIMS

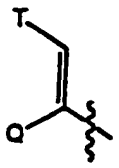
1. A compound having immunosuppressive activity, represented by the formula:



and pharmaceutically acceptable salts thereof,

wherein A is CH_2, oxygen, NH or N-(C1-C4 alkyl);

wherein B and D are independently Ar, hydrogen, (C1-C6)-straight or branched alkyl, (C1-C6)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl or alkenyl that is substituted with a (C5-C7)-cycloalkyl, (C1-C6)-straight or branched alkyl or alkenyl that is substituted with a (C5-C7)-cycloalkenyl, or Ar substituted (C1-C6)-straight or branched alkyl or alkenyl, wherein, in each case, one or two of the CH_2 groups of the alkyl or alkenyl chains may contain 1-2 heteroatoms selected from the group consisting of oxygen, sulfur, SO and SO_2 in chemically reasonable substitution patterns, or



provided that both B and D are not hydrogen;

wherein Q is hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted 5-7 membered cycloalkyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl, O-(C1-C4)-alkenyl and carbonyl;

wherein Ar is selected from the group consisting of phenyl, 1-naphthyl, 2-naphthyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, monocyclic and bicyclic heterocyclic ring systems with individual ring sizes being 5 or 6 which may contain in either or both rings a total of 1-4 heteroatoms independently selected from O, N and S; wherein Ar may contain one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, trifluoromethoxy, (C1-C6)-straight or branched alkyl, (C2-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl, O-(C2-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, 1,2-methylenedioxy, amino, carboxyl and phenyl;

wherein E is (C1-C6)-straight r branched alkyl, (C1-C6)-straight r branched alkenyl,

(C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or branched alkyl or (C1-C4)-straight or branched alkenyl, [(C2-C4)-alkyl or (C2-C4)-alkenyl)]-Ar or Ar (Ar as described above);

wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylmethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an oxygen, sulfur, SO or SO_2 substituent therein;

wherein n is 0 to 3; and

wherein the stereochemistry at carbon position 1 and 2 are R or S.

2. An immunosuppressant compound of Claim 1, having an affinity for FK-506 binding protein.

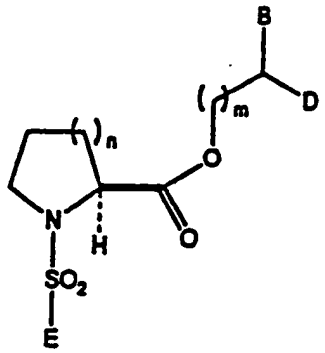
3. An immunosuppressant compound of Claim 1, capable of inhibiting the prolyl peptidyl cis-trans isomerase activity of the FK-506 binding protein.

4. An immunosuppressant compound of Claim 1, having a molecular weight below about 750 amu.

5. An immunosuppressant compound of Claim 4, having a molecular weight below about 500 amu.

6. An immunosuppressant compound of Claim 1, wherein the stereochemistry at carbon position 1 is S.

7. An immunosuppressant compound of Claim 1, wherein J and K are taken together and is represented by the formula:



wherein n is 1 or 2 and m is 0 to 1.

8. An immunosuppressant compound of Claim 7, wherein B is selected from the group consisting of hydrogen, benzyl, 2-phenylethyl and 3-phenylpropyl;

D is selected from the group consisting of phenyl, 3-phenylpropyl, 4-phenoxyphenyl and 4-phenoxyphenyl; and

E is selected from the group consisting of phenyl, 4-methylphenyl, 4-methoxyphenyl, 2-thienyl, 2,4,6-triisopropylphenyl, 4-fluorophenyl, 3-methoxyphenyl, 2-methoxyphenyl, 3,5-dimethoxyphenyl, 3,4,5-trimethoxyphenyl, methyl, 1-naphthyl, 8-quinolyl, 1-(5-N,N-dimethylamino)-naphthyl, 4-iodophenyl, 2,4,6-trimethylphenyl, benzyl, 4-nitrophenyl, 2-nitrophenyl, 4-chlorophenyl and E-styrenyl.

9. A compound having immunosuppressive activity represented by any of the structures shown in Table 1 and having an affinity f r FK-506 binding protein.

10. A composition for use in suppressing an immune response in a mammal, comprising an immunosuppressant compound of Claim 1 having an affinity for FK-506 binding protein and having a molecular weight below about 750 amu, in a physiologically acceptable vehicle.

11. Use of an immunosuppressant compound of Claim 1 having an affinity for FK-506 binding protein and having a molecular weight below about 750 amu in a physiologically acceptable vehicle; for the manufacture of a medicament for use in suppressing an immune response in a mammal.

12. A composition or use according to any one of Claims 10 and 11, wherein the immune response to be suppressed in an autoimmune response or an immune response associated with graft rejection.

13. A composition or use according to any one of Claims 10, 11 and 12, wherein the immunosuppressant compound is represented by the structures shown in Table 1.

14. A composition or use according to any one of Claims 10, 11, 12 and 13, further comprising an immunosuppressant selected from the group consisting of cyclosporin, rapamycin, FK506, 15-deoxyspergualin, OKT3 and azathioprine.

15. A composition or use according to any one of Claims 10, 11, 12, 13 and 14, further comprising a steroid.